

WO 2005/085431

PCT/EP2005/050899

Mutants-P318A.ST25
SEQUENCE LISTING

```

<110> Covalys Biosciences AG
<120> Mutants of 06-Alkylguanine-DNA Alkyltransferase
<130> P318A
<150> EP04405123.3
<151> 2004-03-02
<150> EP04405465.8
<151> 2004-07-22
<160> 48
<170> PatentIn version 3.3
<210> 1
<211> 624
<212> DNA
<213> Homo sapiens
<400> 1
atggacaagg attgtgaaat gaaacgcacc acactggaca gccctttggg gaagctggag      60
ctgtctggtt gtgagcaggg tctgcacgaa ataaagctcc tgggcaaggg gacgtctgca      120
gctgatgccg tggaggtccc agccccgct gcggttctcg gaggtccgga gccctgatg      180
cagtgcacag cctggctgaa tgcctatttc caccagcccg aggctatcga agagttcccc      240
gtgccggcac ttcaccatcc cgttttccag caagagtcgt tcaccagaca ggtgttatgg      300
aagctgctga aggttgtgaa attcggagaa gtgatttctt accagcaatt agcagccctg      360
gcaggcaacc ccaaagccgc gcgagcagtg ggaggagcaa tgagaggcaa tcctgtcccc      420
atcctcatcc cgtgccacag agtgggtctgc agcagcggag ccgtgggcaa ctactccgga      480
ggactggccg tgaaggaatg gcttctggcc catgaaggcc accggttggg gaagccaggc      540
ttgggagggg gctcaggtct ggcaggggcc tggctcaagg gagcgggagc tacctcgggc      600
tccccgcctg ctggccgaaa ctga                                           624

<210> 2
<211> 22
<212> DNA
<213> Artificial Sequence
<220>
<223> Substrate oligonucleotide containing 06-Benzylguanine at position
14

<220>
<221> misc_feature
<222> (14)..(14)
<223> n is 06-benzylguanine
<400> 2
gtggtgggca gctnaggcgt gg                                           22

<210> 3
<211> 33
<212> DNA
<213> Artificial Sequence

```

Mutants-P318A.ST25

<220>
 <223> sense primer for cloning AGT into pGEX
 <400> 3
 cgaaatggat ccatggacaa ggattgtgaa atg 33

<210> 4
 <211> 43
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense primer for cloning AGT into pGEX
 <400> 4
 gcctttgaat tccgtctttg tagtcgtttc ggccagcagg cgg 43

<210> 5
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Sense primer for mutating K125A, T127A, R128A
 <400> 5
 gcaacccgc agccacggca gcagtgggag g 31

<210> 6
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense primer for mutating K125A, T127A, R128A
 <400> 6
 cctccactg ctgccgtggc tgcgggggtt g 31

<210> 7
 <211> 49
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Sense primer for cloning into eukaryotic pNUC vector
 <400> 7
 gatcgagcta ggcgtaccgg tcgccaccat ggacaaggat tgtgaaatg 49

<210> 8
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense primer for cloning into eukaryotic pNUC vector
 <400> 8
 gctagggatc ctacgtttcg gccagcaggc g 31

<210> 9
 <211> 35
 <212> DNA
 <213> Artificial Sequence

Mutants-P318A.ST25

<220>
 <223> Sense primer for mutating Cys 62 to Ala
 <400> 9
 gagccccctga tgcaggctac agcctggctg aatgc 35

<210> 10
 <211> 35
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense primer for mutating Cys 62 to Ala
 <400> 10
 gcattcagcc aggctgtagc ctgcatcagg ggctc 35

<210> 11
 <211> 60
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Sense primer for cloning of AGT mutants into phage-display vector
 <400> 11
 ctactcgcgg cccagccggc catggcggac tacaaagaca tggacaagga ttgtgaaatg 60

<210> 12
 <211> 40
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense primer for cloning of AGT mutants into phage-display vector
 <400> 12
 ggaattcggc ccccgaggcc gcgtttcggc cagcaggcgg 40

<210> 13
 <211> 42
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense primer for cloning AGT truncated after 182 into pGEX
 <400> 13
 gcctttgaat tccgtctttg tagtctccca agcctggctt cc 42

<210> 14
 <211> 50
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Sense primer for randomisation of codons 131-135

<220>
 <221> misc_feature
 <222> (22)..(23)
 <223> n is a, c, g, or t

Mutants-P318A.ST25

<220>
 <221> misc_feature
 <222> (25)..(26)
 <223> n is a, c, g, or t

 <220>
 <221> misc_feature
 <222> (31)..(32)
 <223> n is a, c, g, or t

 <220>
 <221> misc_feature
 <222> (34)..(35)
 <223> n is a, c, g, or t

 <400> 14
 cccaaagccg cgcgagcagt gnnknkgca nnknkggca atcctgtccc 50

 <210> 15
 <211> 26
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense primer for randomisation of codons 131-135

 <400> 15
 tgctcgcgcg gctttggggt tgcctg 26

 <210> 16
 <211> 41
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Sense primer for randomisation of codons 115-116

 <220>
 <221> misc_feature
 <222> (19)..(20)
 <223> n is a, c, g, or t

 <220>
 <221> misc_feature
 <222> (22)..(23)
 <223> n is a, c, g, or t

 <400> 16
 ggagaagtga tttcttacnn bnnbttagca gccctggcag g 41

 <210> 17
 <211> 27
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense primer for randomisation of codons 115-116

 <400> 17
 gtaagaaatc acttctccga atttcac 27

 <210> 18
 <211> 41
 <212> DNA
 <213> Artificial Sequence

Mutants-P318A.ST25

<220>
 <223> Sense primer for randomisation of codons 150-152

<220>
 <221> misc_feature
 <222> (19)..(20)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (22)..(23)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (25)..(26)
 <223> n is a, c, g, or t

<400> 18
 ccgtgccaca gagtggtcnn bnnbnnbgga gccgtgggag g 41

<210> 19
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense primer for randomisation of codons 150-152

<400> 19
 gaccactctg tggcacgg 18

<210> 20
 <211> 35
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Sense primer for mutating G131K, G132T, M134L, R135S

<400> 20
 gcagccacgg cagcagtgaa gacggcactg agtgg 35

<210> 21
 <211> 40
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense primer for mutating G131K, G132T, M134L, R135S

<400> 21
 ggatagggac aggattgcca ctcagtgccg tcttcactgc 40

<210> 22
 <211> 41
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Sense primer for mutating Q115S, Q116H

<400> 22
 gtgaaattcg gagaagtgat ttcttactct cacttagcag c 41

Mutants-P318A.ST25

<210> 23
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for mutating Q115S, Q116H

<400> 23
cctgccaggg ctgctaagtg agagtaagaa atcac 35

<210> 24
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for mutating C150N, S151I, S152N

<400> 24
cgtgccacag agtgggtcaat atcaatggag ccg 33

<210> 25
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for mutating C150N, S151I, S152N

<400> 25
cgtaaccgcc cacggctcca ttgatattga cc 32

<210> 26
<211> 32
<212> DNA
<213> Artificial sequence

<220>
<223> Primer for cloning mutant AGT in pET15b

<400> 26
gtcgcataatg gacaaggatt gtgaaatgaa ac 32

<210> 27
<211> 34
<212> DNA
<213> Artificial sequence

<220>
<223> Primer for cloning mutant AGT in pET15b

<400> 27
gattacggga tccttatccc aagcctggct tccc 34

<210> 28
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for cloning truncated AGT in pAK 100

<400> 28
gcaatggaat tcggcccccaggccgctcc caagcctggc ttccc 45

Mutants-P318A.ST25

<210> 29
 <211> 81
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Sense primer for introducing 12xHis, SbfI and AscI sites

 <400> 29
 ttatccatgg cacatcatca tcatcatcat catcatcatc atcatcatcc tgcaggtata 60
 ggcgcgcccta aaagcttctt a 81

 <210> 30
 <211> 81
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense primer for introducing 12xHis, SbfI and AscI sites

 <400> 30
 taagaagctt ttaggcgcgc ctatacctgc aggatgatga tgatgatgat gatgatgatg 60
 atgatgatgt gccatggata a 81

 <210> 31
 <211> 59
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Sense primer for cloning mutant AGT in pBAD-HisA

 <400> 31
 ggctgcagg tgaaaacctg tacttccagg gtatggacaa ggattgtgaa atgaaacgc 59

 <210> 32
 <211> 45
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense primer for cloning mutant AGT in pBAD-HisA

 <400> 32
 aaaaggcgcg ccggatcctt atcccaagcc tggcttcccc aaccg 45

 <210> 33
 <211> 47
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense primer for cloning wt AGT in pBAD-HisA

 <400> 33
 aacggcgcgc cggatcctta gtttcggcca gcaggcgggg agcccga 47

 <210> 34
 <211> 49
 <212> DNA
 <213> Artificial Sequence

 <220>

Mutants-P318A.ST25

<223> Sense primer for cloning AGTM in pEGFP-Nuc

<400> 34
gatcgagcta gcgctaccgg tcgccaccat ggacaaggat tgtgaaatg 49

<210> 35
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for cloning AGT G160W in pEGFP-Nuc

<400> 35
ccaggcagat ctgtttcggc cagcaggcgg gg 32

<210> 36
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for cloning AGTM in pEGFP-Nuc

<400> 36
ccaggcagat cttcccaagc ctggcttccc caaccggtgg ctttcattg 48

<210> 37
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for cloning beta-Gal in pEGFP-Nuc

<400> 37
catcgtctag attatttttg acaccagacc aac 33

<210> 38
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for cloning beta-Gal in pEGFP-Nuc

<400> 38
gatcgagatc tgggtccgga atgactaaat ctcatcaga ag 42

<210> 39
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for mutation G160W

<400> 39
caactactcc tggggactgg ccgtg 25

<210> 40
<211> 25
<212> DNA
<213> Artificial Sequence

Mutants-P318A.ST25

<220>
<223> Antisense primer for mutation G160W

<400> 40
ccagtcccca ggagtagttg cccac 25

<210> 41
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer for error prone PCR of pAK100 insert, c at position 1 modified with biotin

<220>
<221> misc_feature
<222> (1)..(1)
<223> n is c modified with biotin

<400> 41
ngatccttag acctgaacgc aggtttcccg actggaaag 39

<210> 42
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for error prone PCR of pAK100 insert

<400> 42
gcgtcagggt tacaagttca tggtttacca gcgcctaaag 39

<210> 43
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for amplification of errorprone-PCR product

<400> 43
cgatccttag acctgaacg 19

<210> 44
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for amplification of errorprone-PCR product

<400> 44
gcgtcagggt tacaagttc 19

<210> 45
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Sense primer for saturation mutagenesis AGTM 150-154

Mutants-P318A.ST25

<220>
<221> misc_feature
<222> (18)..(19)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (21)..(22)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (24)..(25)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (27)..(28)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (30)..(31)
<223> n is a, c, g, or t

<400> 45
cgtgccacag agtggctcnk nnknnknnkn nkgtgggcgg ttacgagg

48

<210> 46
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for saturation mutagenesis AGTM 150-154

<220>
<221> misc_feature
<222> (18)..(19)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (21)..(22)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (24)..(25)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (27)..(28)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (30)..(31)
<223> n is a, c, g, or t

<400> 46
cctcgtaacc gccacmnm nnnnnnnnm ngaccactct gtggcag

48

<210> 47
<211> 48
<212> DNA
<213> Artificial Sequence

Mutants-P318A.ST25

<220>
<223> Sense primer for saturation mutagenesis AGTM 31-35

<220>
<221> misc_feature
<222> (17)..(18)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (20)..(21)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (23)..(24)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (26)..(27)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (29)..(30)
<223> n is a, c, g, or t

<400> 47
gcagggtctg cacgaannkn nknknknkn kaaggggacg tctgcagc

48

<210> 48
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense primer for saturation mutagenesis AGTM 31-35

<220>
<221> misc_feature
<222> (19)..(20)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (22)..(23)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (25)..(26)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (28)..(29)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (31)..(32)
<223> n is a, c, g, or t

<400> 48
gctgcagacg tccccttmnn mnnmnnmnnm nnttcgtgca gaccctgc

48